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Mechanismus m6A dráhy na molekulární úrovni a její role v neurologických onemocněních
The m6A pathway at the molecular level and its role in neurological diseases

Bakalářská práce

Školitelka: Mgr. Barbora Černá

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Aneta Švendová

Poděkování:

Tímto bych chtěla poděkovat své školitelce Mgr. Barboře Černé za vstřícnost, ochotu a nekonečnou trpělivost. Také bych chtěla poděkovat Tobymu Wehlovi, mému kamarádovi, za jeho podporu a cenné rady.

Abstrakt

N6-methyladenosin je nejrozšířenější modifikace v eukaryotní messenger RNA. Tato modifikace má reverzibilní charakter, díky souhře dvou skupin enzymů: methyltransferáz a demethyláz. Biologický efekt methylace je zprostředkován tzv. čtecími proteiny. Tento proteinový komplex hraje roli v mnoha molekulárních procesech, jako je splicing, translace a transport. Role této modifikace se objevuje v mnoha závažných neurologických onemocněních, jako jsou Alzheimerova choroba, Parkinsonova choroba, deprese a hyperaktivita s poruchou pozornosti. Cílem této práce je popsat metabolismus m^6A dráhy a její způsob regulace na molekulární úrovni a dát ji do souvislosti s aktuálními neurologickými onemocněními.

Klíčová slova:

mRNA, metabolismus mRNA, N6-methyladenosin, regulace m^6A, FTO, METTL, ALKBH5, neurodegenerativní onemocnění, Alzheimerova choroba, Parkinsonova choroba, depresivní porucha, ADHD

Abstract

N6-methyladenosine is the most abundant modification in eukaryotic messenger RNA. This modification is reversible, thanks to a complex of methyltransferases and demethylases. The biological effects of m^6A are mediated through reader proteins. This complex mechanism of proteins contributes to many molecular processes such splicing, translation and transport. It also plays a role in many serious neurological diseases, such as Alzeheimer's disease, Parkinson's disease, major depressive disorder and attention deficit hyperactivity disorder. The purpose of this thesis is to describe the m^6A pathway, its regulation at the molecular level and to put it into context with neurological diseases of today.

Key words:

mRNA, mRNA metabolism, N6-methyladenosine, m^6A regulation, FTO, METTL, ALKBH5, neurodegenerative disorders, Alzheimer‘s disease, Parkinson's disease, major depressive disorder, ADHD

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List of abbreviations

AD – Alzheimer's disease

ADHD – Attention deficit hyperactivity disorder

ALKBH5 – α-ketoglutarate-dependent dioxygenase alkB homolog 5

Aβ – beta amyloid

BRD4 – bromodomain-containing protein 4

D2R – dopamine type 2 receptor

D3R – dopamine type 3 receptor

eIF3 – eukaryotic translation initiation factor 3

eIF4F – eukaryotic translation initiation factor 4F

EOPD – early onset Parkinson's disease

FTO – fat mass and obesity-associated protein

HPA – hypothalamic-pituitary-adrenal

LOPD – late onset Parkinson's disease

m^{6}A – N6-methyladenosine

m^{6}Am – N^6,2'-O-dimethyladenosine

MDD – major depressive disorder

METTL14 – methyltransferase like 14

METTL3 – methyltransferase like 3

miRNA - microRNA

mRNA – messenger RNA

NFTs – neurofibrillary tangles

NMDAR1 – N-methyl-D-aspartate receptor 1

PD – Parkinson's disease

pre-mRNA – precursor messenger RNA

rRNA – ribosomal RNA

SAM-dependant methyltransferase – S-adenosyl-methionine dependant methyltransferase

SNP – single nucleotide polymorphism

snRNA – small nuclear RNA

SPs – senile plaques

SRSF3 – serine/arginine-rich splicing factor 3

Sxl – sex-lethal gene

WTAP – Wilms' tumour 1-associating protein

YTHDC1 – YTH domain containing protein 1

YTHDC2 – YTH domain containing protein 2

YTHDF1 – YTH domain family protein 1

YTHDF2 – YTH domain family protein 2

YTHDF3 – YTH domain family protein 3

1. Introduction

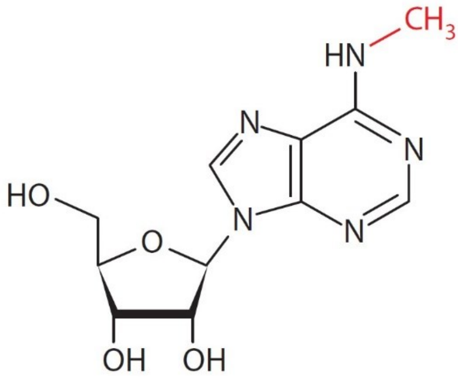
N6-methyladenosine is the most abundant epigenetic modification in messenger RNA (mRNA). It plays a major role in regulating the methylation of mRNA. A complex of methylases and demethylases facilitate the reversibility of this modification, while a group of readers mediate the effects on m^6A. This modification plays an important role in many important biological processes on a cellular level but also influences many fundamental processes of the body as well.

Thanks to the range of roles m^6A modification plays on a molecular level, it could be a key to understanding the impact of molecular pathways on many serious diseases, such as Alzheimer's disease, Parkinson's disease, major depressive disorder, and attention deficit hyperactivity disorder.

The main purpose of this bachelor thesis is to summarize the role of N6-methyladenosine in mRNA methylation, describe the proteins involved in m^6A pathway and highlight the involvement of m^6A in some neurological diseases.

2. N6-methyladenosine

N6-methyladenosine (m^6A) is the most abundant modification in messenger RNA (mRNA). This modification can be also found in noncoding RNAs such as ribosomal RNA (rRNA), small nuclear RNAs (snRNAs) and microRNAs (miRNAs). N6-methyladenosine has been identified in a range of organisms such as yeasts (Bodi et al. 2015), plants (Zhong et al. 2008), insects (G. Zhang et al. 2015), viruses (Krug, Morgan, and Shatkin 1976; C. M. Wei and Moss 1975) and mammals (Dominissini et al. 2012; Xiao Wang et al. 2014). The chemical structure of m^6A can be seen in Figure 1.



m^6A

Figure 1 – Chemical structure of N6-methyladenosine (adapted from Meyer and Jaffrey 2017)

m^6A existence in mRNA was first described in 1974 (Desrosiers, Friderici, and Rottman 1974; Perry and Kelley 1974) and its first function that linked m^6A to mRNA stability was published just four years later (Sommer, Lavi, and Darnell 1978).

N6-methyladenosine is the most abundant post-transcriptional mRNA modification (Cha Mer Wei, Gershowitz, and Moss 1975). It is well known for its reversibility which is facilitated through an interplay between specific groups of proteins called methyltransferases (writers) and demethylases (erasers). The effects of m^6A modification on the fate of decorated mRNA transcripts is mediated by specialized mRNA binding proteins (readers). This complex system of writers, erasers and readers form a regulatory mechanism that influences gene expression in eukaryotic mRNA.

Within decorated mRNA, m^6A is predominantly located at the consensus motif RRACH (R=A/G, H=A/C/U) (N. Liu et al. 2015) around the start codon (5’UTR) and stop codon (3’UTR) (Dominissini et al. 2012; Meyer et al. 2012). This modification does not affect base pairing, but alters RNA folding and structure. These changes may affect the binding of RBP (RNA binding protein) interaction motifs to RNA (N. Liu et al. 2015).

This modification plays a role in regulating many processes in mRNA such as splicing, translation, transport, and stability. These processes will be discussed in later chapters.

3. Contributing proteins of the m^{6}A molecular pathway

Proteins which participate in the m^{6}A pathway can be divided into three main groups. The first group, called writers, consists of enzymes that are responsible for forming the mRNA modification. The second group of enzymes are erasers, which demethylate m^{6}A. The last group are readers. These enzymes are responsible for mediating the effects of m^{6}A methylation.

3.1. Writers

Writers mediate the formation of m^{6}A. The writers complex that is responsible for the majority of m^{6}A sites in mRNA consists of methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14) and is enhanced by Wilms' tumor 1-associating protein (WTAP).

3.1.1. METTL3

Methyltransferase-like 3 (METTL3) is a part of SAM-dependant methyltransferase family. It is located in the nucleus where it acts as the catalytic core of the writer complex. It has already been shown that METTL3 is responsible for the formation of m^{6}A in polyadenylated mRNA (Shimba et al. 1995) and its knockout affects the whole methylation process (Geula et al. 2015).

METTL3 can also act as a reader of methylated transcripts in the cytosol (Lin et al. 2016). It can enhance translation but only when attached to reporter mRNA near the stop codon and if the METTL3-eIF3h (eukaryotic translation initiation factor 3 subunit h) interaction is present. Having said that, a large subset of mRNAs with promoted translation are oncogenic. This includes the bromodomain-containing protein 4 (BRD4) which is linked with human lung tumors. The tumorgenicity of lung cancer cells and its sensitivity to BRD4 inhibition is inhibited by depletion of METTL3 (Choe et al. 2018). METTL3 also plays a role in brain development, more specifically the mammalian cerebellum. The METTL3 knockout express as cerebellar hypoplasia (C. X. Wang et al. 2018).

3.1.2. METTL14

Methyltransferase-like 14 (METTL14) is a protein located in the nucleus that closely interacts with METTL3 by providing structural support and enhancing its enzymatic activity. It was at first classified as another SAM-dependant methyltransferase (Jianzhao Liu et al. 2014). However, that has been disproven by three different studies (Śledź and Jinek 2016; P. Wang, Doxtader, and Nam 2016; Xiang Wang et al. 2016), which showed that METTL14 is not enzymatically active and that it lacks a SAM-binding domain. That makes METTL3 the only protein binding SAM. The role of METTL14 is mainly structural, it binds substrate RNA and positions the methyl group for transfer to adenosine (Śledź and Jinek 2016; P. Wang, Doxtader, and Nam 2016; Xiang Wang et al. 2016).

METTL14 plays a role in many oncological diseases, one example being the upregulation of METTL14 enhancing the migration of cancer cells in breast cancer (Yi et al. 2020). It has also been found to influence insulin secretion and glucose homeostasis, linking it to diabetes (Jun Liu et al. 2019).

3.1.3. WTAP

Wilms' tumor 1-associating protein (WTAP) is a regulatory protein located in the nucleus that binds METTL3 and METTL14 into pre-mRNA processing factors enriched nuclear speckles. It acts as a regulatory subunit that is a crucial part of the METTL3-METTL14 complex and enhances the effectiveness of methylation (as shown in Figure 2). A study confirming the effect of WTAP on the effectiveness of methylation, showed that *Wtap* knockout decreased methylation substantially (Jianzhao Liu et al. 2014). It is also responsible for the catalytic activity of said m^6A methyltransferase in vivo (Ping et al. 2014).

It has been discovered that the levels of METTL3 are closely linked to the WTAP protein homeostasis. Both knockdown and overexpression of METTL3 result in upregulation of WTAP. That being said, the upregulation of WTAP has oncogenic effects only in the presence of functional METTL3, which links the oncogenic effects of WTAP to functional m^6A methylation complex. WTAP is linked closely to many tumors, psoriasis and many other diseases

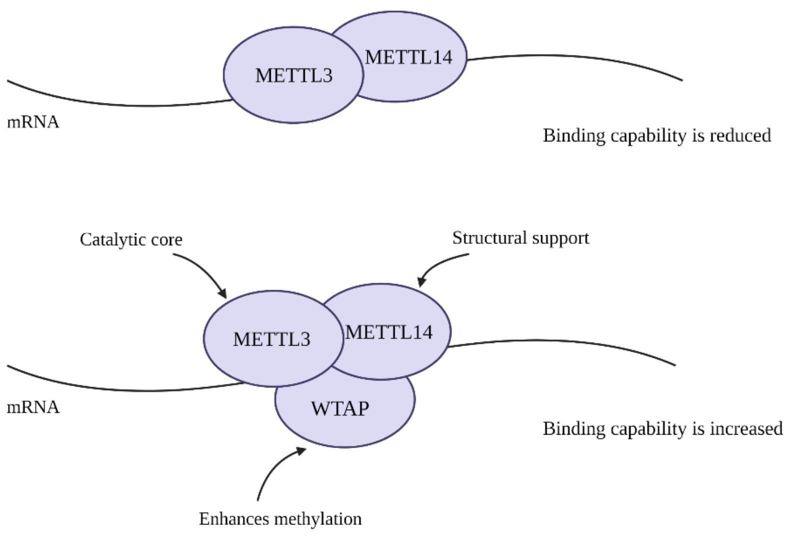


Figure 2 – The WTAP and METTL3/METTL14 interaction is required for effective binding of mRNA (Created with BioRender.com)

3.2. Erasers

Erasers are proteins that demethylate m^6A. The most discussed erasers are fat mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5).

3.2.1. ALKBH5

α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5) is located in the nucleus and influences the levels of m^6A in mRNA through demethylation. It also seems to bind to nuclear RNA (Zheng et al. 2013). ALKBH5 levels do not influence the mice physiology or development but it plays a big role in spermatogenesis (Zheng et al. 2013). The upregulation of ALKBH5 has been linked to diseases such as certain types of cancer (Dixit et al. 2017; S. Zhang et al. 2017) and hypoxia (Thalhammer et al. 2011; C. Zhang et al. 2016).

3.2.2. FTO

Fat mass and obesity-associated protein (FTO) is a protein located in the nucleus that plays an important role in m^6A demethylation. It preferentially binds N^6,2'-O-dimethyladenosine (m^6A_m) and reduces the stability of m^6A_m mRNAs (Mauer et al. 2017). Later studies showed that it also binds m^6A_m in small nuclear RNAs (snRNAs) through which it may influence mRNA splicing (Mauer et al. 2019). FTO binds weakly to m^6A in mRNA and its depletion doesn't seem to substantially influence the levels of m^6A in normal cells (Garcia-Campos et al. 2019; Hess et al. 2013; Mauer et al. 2017).

There have been multiple single nucleotide polymorphisms (SNPs) identified in the *FTO* gene. These SNPs are primarily located in the intron 1 and they affect the expression of neighbouring genes (Smemo et al. 2014; Stratigopoulos et al. 2016). These genetic variants of *FTO* are linked to many diseases such as diabetes and Alzheimer's disease, but this topic will be discussed more thoroughly in later chapters. There have also been links between FTO depletion and levels of m^6A in certain cancers.

3.3. Readers

Readers are proteins that mediate the effects of m^6A methylation and consist of the YTH domain protein family. This family is named after their most recognizable YTH domain which selectively binds m^6A in RNA (Luo and Tong 2014; Theler et al. 2014; C. Xu et al. 2014). Mammalian genomes contain 3 categories of YTH domain-containing proteins: YTHDF family, YTHDC1 and YTHDC2 (also known as DC1 and DC2). Their roles in m^6A molecular pathway are shown in Figure 3.

3.3.1. DF family proteins

The DF family proteins are characterized by 2 domains: YTH domain and large P/Q/N rich low-complexity domain. This group of proteins consists of 3 paralogs: YTHDF1, YTHDF2 and YTHDF3, also known as DF1, DF2 and DF3 (Patil, Pickering, and Jaffrey 2018). This family of proteins is predominantly cytoplasmatic (Xiao Wang et al. 2014). The low-complexity domain is the cause of liquid-liquid phase separation of the DF proteins and the bound m^6A mRNA. This results in mRNA-YTHDF complexes being processed into different compartments like P-bodies, stress granules and neuronal RNA granules. This phase separation is enhanced by mRNAs containing multiple m^6A residues (Ries et al. 2019).

Despite the similarities in structure and same location of the paralogs, earlier studies suggested that different m^6A sites bind different DF paralogs, resulting in limited redundancy of the DF proteins. According to these studies DF1 is responsible for enhancement of mRNA translation, DF2 promotes mRNA degradation and DF3 is responsible for both translation and degradation of mRNA (A. Li et al. 2017; Shi et al. 2017; Xiao Wang et al. 2015). However, a new model for the function of YTHDF family has been proposed in 2020, that suggests all m^6A sites bind all three DF paralogs in a similar level. This is supported by the fact their m^6A-binding properties are basically identical, they share a similar set of associated binding proteins and their subcellular localization is identical throughout the 3 paralogs (Lasman et al. 2020; Zaccara and Jaffrey 2020).

3.3.2. YTHDC1

The YTHDC1 protein has a low complexity domain like the DF proteins and is located in the nucleus. It binds preferentially to non-coding RNA (ncRNA) but it may also bind to mRNA where it influences their splicing and export (Xiao et al. 2016). Notably, it has been linked to mediating the epigenetic effects of XIST, a long non-coding RNA responsible for silencing of gene transcription on one X chromosome in female cells (J. T. Lee 2009). Other processes it has been linked to include pre-mRNA splicing (Xiao et al. 2016) and the export of methylated mRNA from nucleus (Roundtree et al. 2017).

3.3.3. YTHDC2

The role of YTHDC2 protein was unknown for some time. It's YTH domain binds more weakly to m^6A mRNAs than DC1 and the DF family proteins (Wojtas et al. 2017; C. Xu et al. 2015). YTHDC2 protein is localized in the nucleus and cytosol. It has been speculated that DC2 may play a role in mRNA degradation (Kretschmer et al. 2018; Wojtas et al. 2017) and translation initiation (Hsu et al. 2017). The DC2 protein is highly expressed in mouse testes and has an important role in spermatogenesis (Bailey et al. 2017; Hsu et al. 2017; Jain et al. 2017; Wojtas et al. 2017).

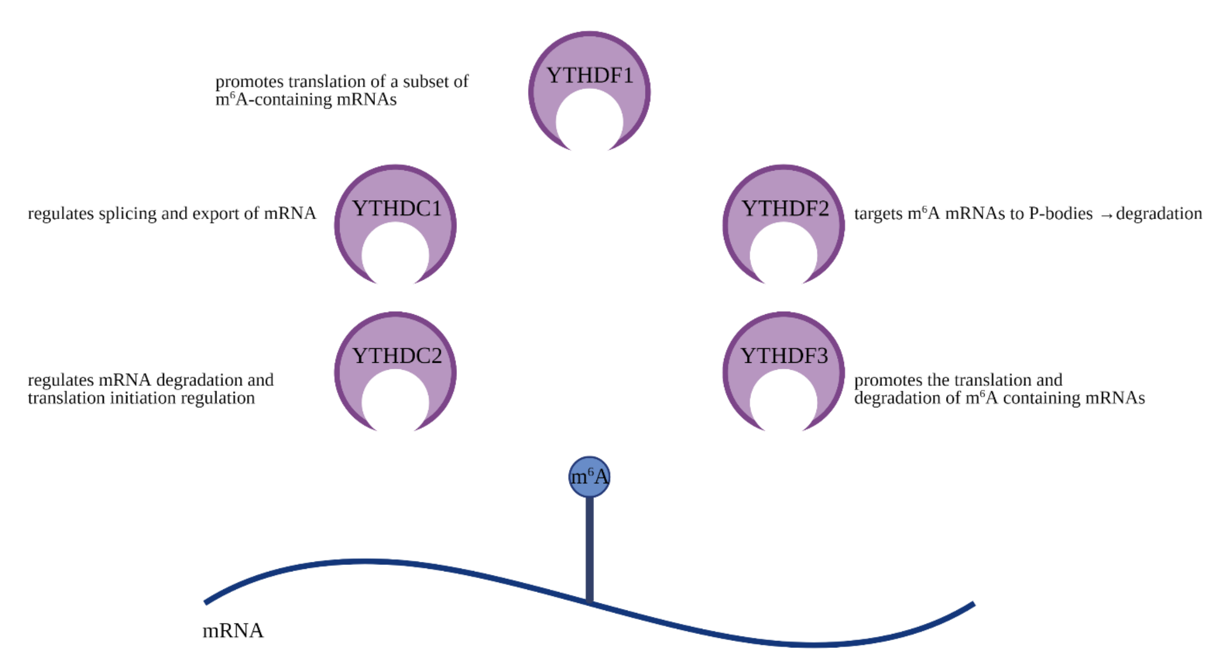


Figure 3 – The role of m^6A reader proteins in m^6A molecular pathway (created with BioRender.com)

4. Roles on a molecular level

m⁶A pathway and its contributing proteins play a big role in molecular processes such as splicing, transport, stability, and translation (as shown in Figure 4).

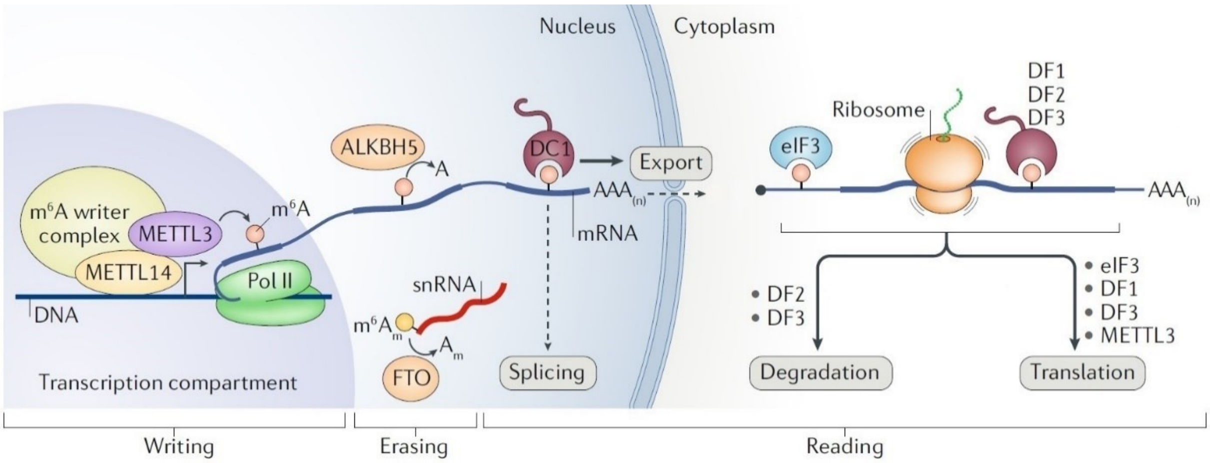


Figure 4 – The life cycle of methylated mRNA (adapted from Zaccara, Ries, and Jaffrey 2019)

4.1. Splicing

Splicing is a process where precursor-messenger RNA (pre-mRNA) is transformed into mature messenger RNA (mRNA). This is accomplished through the removal of non-coding regions called introns leaving only coding regions called exons.

The role of N6-methyladenosine in eukaryotic splicing is still being researched. The strongest evidence can be found in Drosophila, where intronic m⁶A affects splicing of Sex lethal (Sxl) gene, a gene responsible for sex determination in Drosophila (I.U. et al. 2016; Kan et al. 2017; Lence et al. 2016).

The role of m⁶A in mRNA splicing in mammals is less known. The main problem has been mapping out the location of m⁶A in intronic regions. Results of these mappings vary between different research groups with some groups reporting m⁶A enrichments near the exonic 5' splice site (N. Liu et al. 2015), others finding no m⁶A near exonic 5' splice site (Ke et al. 2017). There have also been reports that found m⁶A enrichments in both exonic and intronic regions (Louloupi et al. 2018). Pre-mRNA is stored in nuclear speckles, which are specialized nuclear sites for splicing. The accumulation of METTL3 and METTL14 in nuclear speckles is driven by WTAP protein (Ping et al. 2014). Introns obtain METTL3-binding sites and studies show that *Mettl3* deficiency leads to intron retention and thus disrupts mRNA maturation (Geula et al. 2015). Demethylases FTO and ALKBH5 were also observed in nuclear speckles (Jia et al. 2011).

In mammals, m^6A demethylation through ALKBH5 affects splicing and stability of mRNA in male germ cells in mice. More specifically m^6A erasure mediated through ALKBH5 in spermatogenic cells is needed for correct splicing and the production of longer 3'UTR mRNAs (Tang et al. 2017; Zheng et al. 2013).

4.2. Transport

Another function of m^6A seems to be the enhancement of mature mRNA transport from the nucleus. The transport of methylated mRNA is facilitated through YTHDC1 protein which integrates methylated mRNA in the nuclear transport pathway through interaction with an export adapter that plays a role in mRNA export (SRSF3). YTHDC1 knockdown resulted in accumulation of methylated mRNA in the nucleus and depletion of methylated mRNA in the cytoplasm (Roundtree et al. 2017). Similarly, the depletion of ALKBH5, an eraser protein to m^6A, resulted in an increase of methylation in mRNA in cytoplasm (Zheng et al. 2013), which further validates the role of m^6A in nuclear export.

4.3. Stability

The first well-established function of N6-methyladenosine was its contribution to mRNA instability. A study published in 1978 compared half-lives of mRNAs that contain m^6A and mRNAs without this modification. Their data showed that the half-lives of m^6A containing mRNAs were significantly shorter than those without m^6A (Sommer, Lavi, and Darnell 1978).

According to more recent studies, there are several proteins that contribute to the destabilizing effects of m^6A. One of these proteins is YTHDF2 reader protein which regulates mRNA degradation. However, its depletion influences stability of mRNA only slightly (Xiao Wang et al. 2014). More prominent increase in mRNA half-lives can be seen in cells with METTL3 depletion (Ke et al. 2017).

4.4. Translation

N6-methyladenosine is linked to translation upregulation through different specific mechanisms.

The first mechanism is mediated through an m^6A reader YTHDF1, also known as DF1. YTHDF1 is the first of YTH proteins to be linked to translation. YTHDF1 knockdown in HeLa cells resulted in reduction of translation efficiency YTHDF1 targeted mRNA (Xiao Wang et al. 2015). It has been implied that YTHDF1 facilitates translation through binding eukaryotic translation initiation factor 3 (eIF3), a multiprotein complex that binds the small ribosomal subunit to mRNAs (Xiao Wang et al. 2015).

The second mechanism is a direct interaction between m^6A and eukaryotic initiation factor 3 eIF3. The majority of translations requires eukaryotic initiation factor 4F (eIF4F) that recognizes the 5’ cap of mRNA and recruits eIF3 in mammals. m^6A in 5’ UTR bind eIF3 and induce translation initiation without the presence of eIF4F (A. S. Y. Lee, Kranzusch, and Cate 2015). This suggests that the presence of m^6A in 5’ cap may be a way for translation to start even if eIF4F protein is damaged or missing.

The third mechanism utilizes METTL3. Although METTL3 is predominantly located in the nucleus (especially nuclear speckles), it was also detected in cytoplasm, where it binds eIF3 hence activating translation (Choe et al. 2018; Lin et al. 2016).

It was also shown that m^6A modification within coding regions can affect translation-elongation dynamics via destabilizing pairing between codons and tRNA anti-codons (Choi et al. 2016).

5. Overall impact

Outside of its role in mRNA life cycle, m^6A has an overall impact on biological functions of eukaryotic organisms. m^6A plays a role in fundamental processes of the body such as metabolism, cell differentiation, the development of central nervous system and much more.

The central nervous system (CNS) contains much higher levels of m^6A than other organs (Lence et al. 2016). m^6A modification has already been detected in various types of neural cells such as granule neuronal cells, Purkinje cells, Bergmann glia cells, neuronal cells, oligodendrocytes, and astrocytes, where it affects their function and maturation. It has already been shown that m^6A is highly enriched in neural synapses (76,8% of mouse postsynaptic genes are methylated) and affects synaptic transmission and axon guidance in neurons (Chang et al. 2017). m^6A is also associated with myelination via oligodendrocyte maturation (H. Xu et al. 2020), neurogenesis (L. Li et al. 2017; Yoon et al. 2017), gliogenesis (J. Wang, Sha, and Sun 2021) and proliferation (Batista et al. 2014; Y. Wang et al. 2014).

Brain development is strongly affected by m^6A pathway. The enrichment of methylation varies during this process in brain structures and opens the possibility of spatio-temporal regulation (Chang et al. 2017; Meyer et al. 2012). The key proteins of m^6A pathway play pivotal roles in brain developmental processes and its disruption leads to embryonic lethality, morphological abnormalities and impaired learning and memory (L. Li et al. 2017; Peters, Ausmeier, and Rüther 1999; Yoon et al. 2017).

It was also shown that important metabolic processes are regulated via m^6A pathway (Kobayashi et al. 2018). This links m^6A and its regulatory proteins to metabolic diseases such as obesity and diabetes. Various studies have already discussed FTO demethylase as a protein with an impact on lipid and glucose metabolism (Poritsanos, Lew, and Mizuno 2010; Speakman 2010). FTO overexpression is linked to decreased levels of m^6A in type 2 diabetes patients (Shen et al. 2015). Other m^6A regulatory proteins that contribute to metabolism are METTL3, METTL14 and WTAP (Kobayashi et al. 2018).

6. Neurological diseases

The m^6A modification plays a role in many serious human diseases such as different types of cancer, neurological diseases, cardiovascular diseases, diabetes, and other metabolic disorders. This chapter will discuss some of the neurological diseases like Alzheimer's disease (AD), Parkinson's disease (PD), major depressive disorder (MDD) and attention deficit hyperactivity disorder (ADHD), which have already been associated with m^6A pathway.

6.1. Alzheimer's disease

Alzheimer disease (AD) is one of the most frequent neurodegenerative diseases in today's world. It is the most common cause for dementia and is slowly becoming one of the biggest problems our society has to deal with. As the general lifespan of the world's population increases, so does the risk of AD. Alzheimer's disease causes memory loss, disorientation, changes in behaviour and gradual loss of bodily functions. There is no known cure and even the mechanism of this disease is still unclear.

The pathophysiological signs of AD in the patient's brain are senile plaques (SPs) and neurofibrillary tangles (NFTs). Senile plaques are extracellular protein deposits that disrupt the communication between cells. These plaques are made of beta amyloid (Aβ) (Masters et al. 1985). Neurofibrillary tangles are intracellular protein formations, that are made of the hyperphosphorylated tau proteins. Tau proteins are responsible for the stability of microtubules and when these proteins get hyperphosphorylated, the microtubules can fall apart (Weingarten et al. 1975). The specific mechanics of AD pathogenesis is still being researched, but the most common opinion is that changes in synaptic function are involved (Henstridge, Pickett, and Spires-Jones 2016).

In recent years, a link has been discovered between Alzheimer's disease and m^6A pathway disruption. An experiment on AD animal model revealed that RNA m^6A methylation was elevated in AD mouse model, especially in the cortex and hippocampus. The same study also reported elevated METTL3 expression and decreased FTO expression in AD mice (Han et al. 2020). Another study has shown that m^6A influences aging of the mammalian brain through regulation of mRNA expression levels of transcripts that regulate aging. This study also reported decreased levels of m^6A methylation of Alzheimer related transcripts, which further confirms the role of m^6A modification in Alzheimer's disease (Shafik et al. 2021).

The protein which has been strongly associated with AD is demethylase FTO. It has been shown that some variants of *Fto* increase the risk of developing AD quite significantly. In the first study linking *Fto* and Tau phosphorylation, *Ft*o knockdown decreased the phosphorylation of Tau proteins (Jason T Fong 2013). Another study supported the engagement of FTO with Tau phosphorylation, thus confirming the role of FTO in AD. This study showed that Tau phosphorylation levels were reduced in *Fto* knockdown neurons isolated from 3xTg AD mice. Similarly, the *Fto* overexpression had an opposite effect on Tau phosphorylation (H. Li et al. 2018). Genetic variations of FTO and obesity have been linked to reduced brain volume (Ho et al. 2010) and verbal fluency (Benedict et al. 2011). The rs9939609SNP variant of *Fto* increases the risk of AD by 1.6 fold (Keller et al. 2011). Another study reported that genetic variations in introns 1 and 2 of *Fto* gene may contribute to greater risk of AD (Reitz et al. 2012). The research of genetic variants of *Fto* gene could open new possibilities for the treatment of AD.

6.2. Parkinson's disease

Parkinson's disease (PD) is another neurodegenerative disease that mainly affects the motor functions. This disease usually starts off slowly with tremors, difficulty walking and slowness of movements. Other symptoms may include cognitive issues, hallucinations, and behavioural alterations. The mechanism of this disease is still unknown and there is currently no cure, only treatments that focus on alleviating the symptoms. There are two types of Parkinson's disease based on the age the motor symptoms appear. Early onset Parkinson's disease (EOPD) appears before 50 years of age and late onset Parkinson's disease (LOPD) after. Genetics seems to play a big role in development of PD. Another contributing factor could be the environment.

The pathology of PD is characterized by the death of dopaminergic neurons. Dopaminergic neurons make up a substantial part of substantia negra, a basal ganglia structure in the midbrain, that plays an important role in movement and motor skills. The mechanism that causes neuron's death in PD patients is currently unknown. The pathophysiology of PD is characterized by aggregation of alpha-synuclein protein called Lewy bodies inside the nerve cells (Braak et al. 2003). These aggregations of proteins are not directly responsible for neuron death but contribute otherwise to the disruption of neurotransmitter pathway.

In 2013, a study uncovered a role of FTO in dopaminergic midbrain signaling. Conventional and conditional *Fto* deficient mice had impaired sensitivity of dopamine type 2 receptor (D2R) and dopamine type 3 receptor (D3R) (Hess et al. 2013). Another study showed that decrease of m^6A in dopaminergic neurons could activate the expression of N-methyl-D-aspartate receptor 1

(NMDAR1) which could result in increased dopaminergic cell death (Chen et al. 2019). Overall, these studies suggest that m^6A modification might play a bigger role in the mechanism of PD pathophysiology.

6.3. Major Depressive Disorder

Major depressive disorder (MDD) is a recurrent mental disorder that is characterized by extensive periods of low mood, loss of interest in normally enjoyable activities, lack of motivation and low energy. This disorder is becoming more prevalent in recent years, most probably due to the slow destigmatization of mental illnesses in the public sphere. This disorder can heavily affect personal and working life of a patient. Other psychological problems also occur often with MDD, such as anxiety. People with ADHD have a greater risk of developing MDD in their life.

Many factors play a role in developing MDD, such as genetics, environmental factors, and psychological factors. The pathophysiological mechanisms of MDD are currently unknown, but there have been links to inflammation (Vogelzangs et al. 2012), hypovitaminosis D (Milaneschi, Hoogendijk, et al. 2014), hypothalamic-pituitary-adrenal (HPA) axis activity (Vreeburg et al. 2009), and neurotrophic grow (Molendijk et al. 2011).

FTO polymorphism has been linked to the pathogenesis of MDD. Especially the rs9939609 variant of *FTO* might play a big role in the development of MDD (Milaneschi, Lamers, et al. 2014; Samaan et al. 2013). However, both of these studies have come to a different result. Samaan et al. reports that *FTO* rs9939609 variant decreases the risk of MDD, while Milaneschi et al. found a positive association between this variant and MDD. This is most probably caused by atypical subtype of MDD in Milaneschi et al. study. Another study tried to replicate Samaan et al. study but could not find the link between rs9939609 *FTO* variant and MDD (Du et al. 2015). However, this study suggests a link between SNP rs12936694 variant of *ALKBH5* and MDD. Newer study reported a impaired regulation of m^6A in MDD patients after glucocorticoid stimulation (Engel et al. 2018). Overall, the research on m^6A pathway link to MDD is still in the early stages. The reason why so many of these studies seem to contradict each other are probably caused by the heterogeneity of MDD.

6.4. Attention deficit hyperactivity disorder

Attention deficit hyperactivity disorder (ADHD) has also been suggested to be affected by m^6A pathway. It is neurodevelopmental disorder that is characterized by impulsive behaviour, inability to stay attentive and hyperactivity. In children ADHD often results in poor school performance. Adults with ADHD usually develop coping mechanisms that help them overcome their impairment. People with ADHD have stronger predisposition for substance abuse and other mental disorders. The majority of people diagnosed with ADHD are males since the early studies mostly focused on Caucasian males. Most females show much different signs of ADHD, they are much less disruptive and "mask" better (Gershon 2002).

The factors involved in development of ADHD are genetics, environmental and social factors. Other factors include a number of factors during pregnancy and birth. Trauma to the head or brain in the early age can also trigger development of ADHD (Millichap 2008). Genetics play the biggest role with about 70% of ADHD cases in children being determined by genetic predisposition (Faraone and Mick 2010). The pathophysiological signs of ADHD in children are decreased brain volume (Krain and Castellanos 2006) and disruption in certain neurotransmitter systems (Volkow et al. 2009).

The role of m6A pathway in ADHD has not been completely clear so far. It was shown that *Fto* duplication (due to 16q trisomy) leads to mental retardation and ADHD onset (Van Berg et al. 2010). Two FTO variants, which affect ADHD have been described so far and interestingly. they decrease the risk for symptoms of ADHD in children (Choudhry et al. 2013; Velders et al. 2012).

7. Conclusion

N6-methyladenosine is the most abundant mRNA modification in eukaryotic organisms. Thanks to its reversibility it plays a dynamic role in the mRNA methylation. A complex mechanism of writer, eraser and reader proteins affect many processes in the cell life cycle and also biological functions of the body. This links the modification to many serious diseases ranging from metabolic, cardiovascular, neurological diseases, and many types of cancer.

The prevalence of m^6A modification in the brain links this powerful modification to many neurological diseases that are incurable at this time. This makes the m^6A pathway and its contributing proteins a perfect candidate for further research. Genetic variants of these contributing proteins could be the key to understanding mechanisms of development of Alzheimer's disease and Parkinson's disease.

In conclusion, there is still a lot we do not know about this modification and the proteins involved in its pathway. Further research could uncover new roles of m^6A pathway in development of diseases and could help us understand the mechanisms of certain neurological diseases.

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Figure 2 and **Figure 3** were created with BioRender.com

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